NITRIC OXIDE SEQUESTRANT

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FIELD OF THE INVENTION

The present invention provides a method of sequestering nitric oxide from the bloodstream, endothelium or tissues of mammals by administering a cobalamin to such mammals. The present invention further provides a method of treating diseases in a mammal characterized by elevated nitric oxide levels in the bloodstream, endothelium or tissues of such mammals by administering a therapeutic dose of a cobalamin. In particular, the present invention describes a method of treating mammals with sepsis by administering a therapeutic dose of hydroxocobalamin. A method of reducing pathologic nitric oxide levels in mammals by administering a therapeutic dose of a cobalamin to sequester the excess nitric oxide is also described by the present invention. A method of alleviating systemic hypotension in a septic patient is further provided by the invention. A pharmaceutical composition comprising a cobalamin in a concentration ranging from 0.5 to 50 mg composition/kg body weight for mammals is also provided.

BACKGROUND OF THE INVENTION

Furchgott and Zowadzki discovered that vascular endothelium played a critical role in the regulation of vascular tone through the release of endothelium-derived relaxing factor (EDRF). Furchgott et al. (1980) *Nature* 280:373–376. In 1988, Palmer et al. concluded that EDRF was actually nitric oxide when they observed that the release of nitric oxide from the endothelial cells occurred in amounts sufficient to account for the biological activity attributed to EDRF. Palmer et al. (1987) *Nature* 327:524–526. It is now known that nitric oxide regulates a continuous vasodilator tone and thereby maintains normal homeostatic blood pressure.

A number of pathological conditions are characterized by excess nitric oxide production. Such diseases include the systemic inflammatory response syndrome (SIRS) including sepsis and septic shock; endotoxemia; GI inflammatory diseases such as ileitis, colitis and Crohn's Disease; chronic inflammatory disease; autoimmune disorders; and rheumatoid arthritis. Moncada et al. (1993) *N. Engl. J. Med.*, 329: 2002–2012. Most recently, pertussis (whooping cough) was found to be characterized by an excess production of nitric oxide. Leff (1994) *Bioworld Today*, 5:1. Various treatments, such as treating cancer with cytokines, can also lead to elevated nitric oxide levels.

The importance of understanding the role of nitric oxide in these pathological conditions is evident from the incidence and mortality rates associated with these diseases. For example, the Centers for Disease Control estimates that sepsis occurs in approximately 500,000 patients in the United States and 400,000 patients in the European community annually and is associated with a 35% mortality rate. It is the most common cause of death in noncoronary, intensive care units. With regard to GI inflammatory conditions, the Crohn's and Colitis Foundation of America estimates that 1 million people suffer from ulcerative colitis in the United States with 15,000 new cases reported annually. Further, approximately 2 million people are reported to have rheumatoid arthritis.

Nitric oxide is synthesized in mammalian cells from the amino acid L-arginine by a family of enzymes, the nitric oxide synthases, via the L-arginine-nitric oxide pathway.

Moncada et al. (1993) *N. Engl. J. Med.*, 329:2002–2012. The production of nitric oxide via the nitric oxide-arginine pathway begins when a guanidine nitrogen of L-arginine undergoes a five-electron oxidation to yield the gaseous radical nitric oxide via an N^{ω}-hydroxyl-L-arginine intermediate. NADPH (nicotine adenine diphosphonucleotide, reduced) donates two electrons for the formation of this intermediate and one electron for its further oxidation. Both steps are catalyzed by nitric oxide synthase. In addition to the gaseous radical nitric oxide, L-citrulline is also produced. Molecular oxygen is incorporated into both the L-citrulline and the nitric oxide formed. Tetrahydrobiopterin is required for the oxidation of the intermediate, N^{ω}-hydroxyl-L-arginine, to L-citrulline. The amount of tetrahydrobiopterin required is substoichiometric with respect to the nitric oxide generated, provided that tetrahydrobiopterin can be regenerated from its oxidized form, quinonoid dihydrobiopterin.

Two distinct isozymes of nitric oxide synthase have been identified and include constitutive nitric oxide synthase (cNOS) and inducible nitric oxide synthase (iNOS). These isoforms differ in primary structure, cofactor requirements, tissue distribution and activation state.

Under basal conditions, endothelium-derived nitric oxide is produced by cNOS, a calcium- and calmodulin-dependant nitric oxide synthase. Constitutive nitric oxide synthase is controlled by cell surface receptors and can be activated by a variety of vasodilators including acetylcholine, bradykinin, histamine and adenosine. This enzyme is always present in the vascular endothelium of mammals. The interaction of acetylcholine or bradykinin with their receptors on vascular endothelium results in production of intracellular calcium which stimulates cNOS. The nitric oxide formed from L-arginine diffuses to nearby smooth muscle cells where it stimulates the soluble guanylate cyclase, resulting in enhanced synthesis of cyclic guanosine monophosphate (cGMP). The cGMP formed causes smooth muscle cells to relax. The formation of cGMP regulates physiological vascular tone, blood pressure and tissue perfusion by mediating endothelium-dependent relaxation and neural transmission.

The second isoform of nitric oxide synthase, inducible nitric oxide synthase, is calcium-independent and is not controlled by receptor-dependent mechanisms. This enzyme is induced in endothelial, vascular smooth muscle and phagocytic cells by endotoxins and various cytokines. When these cytokines interact with their respective receptors, calcium-independent nitric oxide synthase is induced. The induction of this enzyme causes prolonged nitric oxide synthesis, resulting in sustained activation of soluble guanylate cyclase. Continuous production of cGMP ultimately leads to prolonged smooth muscle relaxation, reduced responsiveness to vasoconstricting drugs, and possible tissue damage.

The synthesis of nitric oxide from L-arginine occurs in numerous cells and tissues. Examples of cells which produce nitric oxide include: neutrophils, megakaryocytes, Kupffer cells, macrophages, endothelial cells, hepatocytes, murine fibroblasts and EMT-6 cells. Examples of tissues that generate nitric oxide include: vascular smooth muscle, the brain, the adrenal gland, endocardium, peripheral and sensory nerves and the myocardium. Moncada et al. (1993) *N. Engl. J. Med.*, 329: 2002–2012.

The role of nitric oxide in the initiation of sepsis in a mammal is known in the art. Sepsis is defined as the presence of pathologic microorganisms or their toxins in the blood or other tissues of a mammal. Septic shock is shock